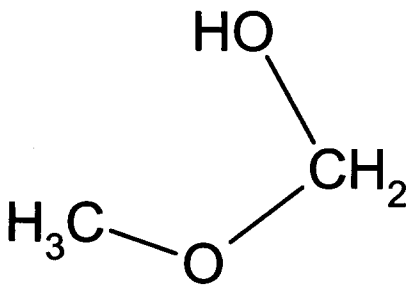
201-15973A

Methoxymethanol

CAS Number 4461-52-3



USEPA HPV Challenge Program Submission

Revised Test Plan

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Executive Overview

Methoxymethanol (CAS Number 4461-52-3) is a transient equilibrium species (or compound) found in mixtures of formaldehyde and methanol, which may also contain water. It is produced from a highly concentrated hydrated formaldehyde compound by dissolution in methanol. The primary use of these equilibrium mixtures is as a chemical intermediate in the manufacture of urea formaldehyde and melamine formaldehyde resins, which are used for coatings, adhesives, molding compounds and similar applications. These equilibrium mixtures offer advantages over using aqueous solutions of formaldehyde for many applications.

The equilibrium constants for the reaction of formaldehyde with methanol to produce methoxymethanol and the reaction of formaldehyde with water to produce methylene glycol are published and have been verified by additional independent publications. Likewise, rate constants of formation and degradation of methoxymethanol and methyleneglycol have been published. It is possible to use these constants to accurately calculate the quantities of methoxymethanol and methylene glycol in ternary mixtures of formaldehyde, methanol and water where water is the predominant species. These calculation are shown in this document and lead to the conclusion that the quantity of methoxymethanol itself is negligible at any relevant concentration regarding risk assessments for environmental or health effects.

Methoxymethanol (CAS Number 4461-52-3) is not produced as a pure chemical entity as it is an unstable species that has no known application in commerce other than as an incidental species in methanolic formaldehyde. Celanese Ltd., the sponsor of this chemical in the U.S. EPA HPV Program markets a commercial product known as Methyl Formcel ® that has the nominal composition formaldehyde (55%), methanol (35%) and water (10%).

The physicochemical properties of methoxymethanol are not well established since it is not isolatable under ambient conditions. Methoxymethanol can be observed spectroscopically (e.g. nmr and ms) but is not stable enough for convenient determination of bulk physicochemical properties. The commercial product boils at between 90 and 95°C, and has a vapor pressure of 40 hPa at 40° C. Methoxymethanol, as a discrete chemical entity, is predicted to be miscible with water and have a log $K_{o/w}$ of –1.4. Physicochemical properties of formaldehyde and methanol are also described in the document as they are relevant to hazard and risk assessment of commercial product.

Methoxymethanol can be considered readily biodegradable as both methanol and formaldehyde are readily biodegradable and the half-life of the methoxymethanol molecule in water is six minutes or less. Methoxymethanol, methanol and formaldehyde undergo relatively rapid indirect photolysis in the atmosphere with half-life estimated to be less than 16 hours. EQC Level III calculations indicate that methoxymethanol, methanol and formaldehyde distribute preferentially to water and followed closely by soil.

Fish, invertebrates and algae are all predicted to be relatively insensitive to toxicity by the methoxymethanol molecule when considered as a neutral organic species; however, due to rapid hydrolysis in water, the toxicity of formaldehyde to aquatic species is considered the relevant way to evaluate the aquatic toxicity of methoxymethanol. Approximate LD_{50} and ED_{50} values have been calculated for the Celanese commercial product based on formaldehyde content to be 45, 10.5 and 11.5 mg/L for fish, invertebrates and green algae, respectively.

The acute oral toxicity in rats has been determined for a "methoxymethanol" test material that was 46.7% methoxymethanol with 44.93% methanol. The study results indicate the oral LD_{50} values for male or female rats were 1269 mg/kg for males (95% confidence limit: 981-1636 mg/kg), and 1451 mg/kg for females (95% confidence limit: 1059-2000 mg/kg). These values suggest that hydrolysis to formaldehyde is the critical step in the acute toxicity of methoxymethanol. The document compares this result with the acute oral toxicity formaldehyde and methanol.

An OECD 422 guideline repeated dose with reproductive and developmental screen has been conducted on a "methoxymethanol" test material that was 46.7% methoxymethanol with 44.93% methanol. Effects appear to be primarily at the site of contact and related to the irritant properties of the test substance. The GI tract is identified as the target organ and biochemical and hematologic changes are considered secondary to gastric ulceration and subsequent loss of blood. The LOAEL was determined to be 60 mg/kg-day for males and 300 mg/kg-day for females. The NOAEL are considered to be 12 mg/kg-day for males and 60 mg/kg-day for females.

An Ames test and an in vitro chromosome aberration study have been conducted on a "methoxymethanol" test material that was 46.7% methoxymethanol with 44.93% methanol. It was found to have genotoxic activity in both assays. A discussion of the genotoxicity of methanol and formaldehyde is provided in the document.

Reproductive and developmental toxicity were assessed in the OECD-422 conducted on a "methoxymethanol" test material that was 46.7% methoxymethanol with 44.93% methanol. Although the OECD-422 results did not indicate any reproductive or developmental hazard from this mixture, developmental toxicity studies in rodents have shown specific developmental toxicity of methanol at high doses. Differences in methanol metabolism between humans and rodents indicate that these high-dose developmental effects in rodents are not relevant to man. A thorough discussion of methoxymethanol, methanol and formaldehyde is given in the document detailing the metabolic differences and evaluating the developmental hazard to man.

With regard to the parameters specified in the EPA HPV Challenge program, the available information fills all of the requirements for physicochemical parameters, environmental fate, and toxicity information. No additional testing is recommended.

Testing Plan and Rationale

Testing Plan in Tabular Format

CAS Number 4461-52-3 Methoxymethanol	Information Available?	OECD Study ?	GLP Study?	Supporting Information?	Estimation Method?	Acceptable?	Testing Recommended?	
HPV Endpoint								
Physical Chemical								
Melting Point	Y	N	N	N	N	Y	N	
Boiling Point	Y	N	N	Y	N	Y	N	
Vapor Pressure	Y	N	N	Y	N	Y	N	
Partition Coefficient	Y	N	N	Y	N	Y	N	
Water Solubility	Y	N	N	Y	N	Y	N	
Environmental & Fate								
Photo-Degradation	Y	N	N	Y	Y	Y	N	
Water Stability	Y	N	N	Y	Y	Y	N	
Transport	Y	N	N	N	Y	Y	N	
Biodegradation	Y	N	N	Y	N	Y	N	
Ecotoxicity								
96-Hour Fish	Y	N	N	Y	N	Y	N	
48-Hour Invertebrate	Y	N	N	Y	N	Y	N	
96-Hour Algae	Y	N	N	Y	N	Y	N	
Toxicity								
Acute	Y	Y	Y	Y	N	Y	N	
Repeated Dose	Y	Y	Y	Y	N	Y	N	
Genetic Toxicology *in vitro*	Y	Y	Y	Y	N	Y	N	
Genetic Toxicology *in vivo*	Y	Y	Y	Y	N	Y	N	
Reproductive	Y	Y	Y	Y	N	Y	N	
Developmental	Y	Y	Y	Y	N	Y	N	

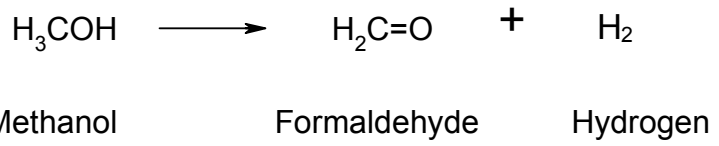
Introduction

Methoxymethanol (CAS Number 4461-52-3) is a transient equilibrium species (or compound) found in mixtures of formaldehyde and methanol, which may also contain water. Methoxymethanol (CAS Number 4461-52-3) is not produced as a pure chemical entity as it is an unstable species that has no known application in commerce other than as an incidental species in chemical mixtures that contain methanol and formaldehyde.

It is produced from a highly-concentrated hydrated formaldehyde compound by dissolution in methanol. The primary use of these equilibrium mixtures of methanol and hydrated formaldehyde is as a chemical intermediate in the manufacture of urea formaldehyde and melamine formaldehyde resins, which are used for coatings, adhesives, molding compounds and similar applications.

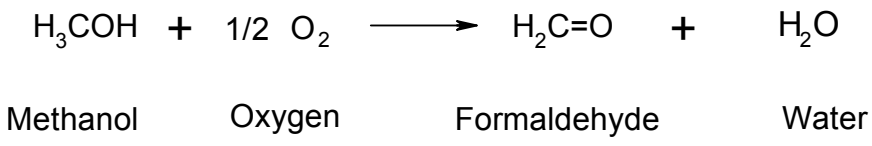
Celanese Ltd., the sponsor of this chemical in the U.S. EPA HPV Program markets a commercial product known as Methyl Formcel ® that has the nominal composition formaldehyde (55%), methanol (35%) and water (10%). The production of nominal quantities of methoxymethanol is inseparable from the commercial production of formaldehyde since methoxymethanol is produced by the reaction of formaldehyde with methanol and all commercial processes for formaldehyde production have some residual methanol. Formaldehyde is produced from methanol by two processes using either a silver catalyst or a metal oxide (iron-molybdate) catalyst. Each process is practiced in a number of variations.

The silver catalyst process is a combination oxidation-dehydrogenation of methanol and is commonly represented by the following chemical equation:



This is an exothermic reaction and the reaction is typically quenched with water and steam is recovered as a by-product. Metallic silver in the form of gauze or crystals is used as the catalyst.

The second important production method, the metal oxide process, involves the catalytic oxidation of methanol by a mixed oxide catalyst containing iron and molybdenum with other metals (e.g. chromium) often used as catalyst promoters. The reaction is typically represented as:

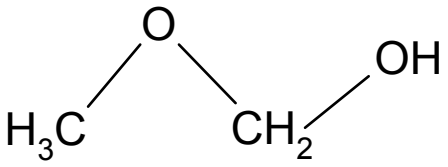


The primary use of methanolic formaldehyde (methoxymethanol) is as a chemical intermediate in the manufacture of urea formaldehyde and melamine formaldehyde resins, which are used for coatings, adhesives, molding compounds and similar applications. These equilibrium mixtures of methanol and hydrated formaldehyde offer advantages for many applications over using aqueous solutions of formaldehyde as they contain more formaldehyde per pound than aqueous preparations, they contain less water, they offer enhanced stability and they can be stored at lower temperatures than aqueous formaldehyde.

Aqueous solutions of formaldehyde require an inhibitor to prevent excessive polymerization at low storage temperatures. Methanol is most commonly used for this purpose and inhibited grades of aqueous formaldehyde usually contain 7-15% methanol. Therefore, the actual quantity of methoxymethanol in commerce is the total of the methoxymethanol contained in the methanol hydrated-formaldehyde mixtures used in industry, and the methoxymethanol contained in commercial methanol-inhibited aqueous formaldehyde.

The TSCA 1975-77 inventory reporting stated that 50,000,000 to 100,000,000 pounds of methoxymethanol were produced by one plant. Current production volumes of Methyl Formcel® are in the range of 1,000,000 to 10,000,00 pounds. Other manufactures use formaldehyde-methanol solutions as a site limited intermediate and their production level is not known. If the stabilized grades of aqueous formaldehyde are considered the annual incidental production of methoxymethanol is much greater. Although the quantity of stabilized formaldehyde produced in the US is not published it can be estimated from the annual capacity for formaldehyde production of 5,648,000 metric tons, which operates at about 85% of capacity to produce about 5,000,000 metric tons of total formaldehyde. Much of this is captive and not shipped as inhibited formaldehyde. A rough estimate is that 10% of formaldehyde produced is shipped as methanol-inhibited aqueous solutions and of this, 15 mole % is in the form of methoxymethanol (*vide post*); therefore, it is possible that 150,000,000 pounds of methoxymethanol is produced, shipped and utilized in the U.S. annually as an incidental compound in formaldehyde/methanol/water ternary mixtures.

The structure of methoxymethanol is shown below:



Methoxymethanol

Methoxymethanol is also known as:

- Formaldehyde methyl hemiacetal
- Hemiformal
- Methanol, hemiformal
- Methanol, methoxy- (8CI, 9CI)
- Methyl hemiformal

The primary application of methoxymethanol is as a chemical intermediate in the manufacture of various urea formaldehyde and melamine formaldehyde resins. It is a more effective means of delivering formaldehyde to a reaction, as the quantity of formaldehyde is greater in methanol-hydrated-formaldehyde preparations than in water solutions of formaldehyde. In addition, methanol-hydrated-formaldehyde preparations have significant advantages if water is undesirable in the reaction mixture. The resins that are produced from methanol-hydrated-formaldehyde find application in a broad array of uses including coatings, adhesives, and molding compounds (1).

Celanese has a total of approximately 15–20 operators that work in areas where there could possibly be methoxymethanol exposure as a side-stream. Exposure in industrial applications is limited by process controls and protective equipment. Manufacture of this material is in a closed system and the only significant exposure is in sampling and possible rework of off-specification materials. The only other potential for exposure is in the event of a spill or upset. As the permissible exposure limit falls under the OSHA Formaldehyde Standard it is treated as a regulated chemical and when there is sampling, clean up, or rework appropriate engineering controls, work practices, and Personal Protective Equipment are specifically required for each task.

Transportation of methanol-hydrated-formaldehyde preparations is primarily in rail cars and tank trucks as the applications typically consume large quantities of this mixture and it can be conveniently piped from the transportation vehicle to storage tanks. Since the material is primarily transported by pipe in a chemical plant, and since the material falls under the OSHA Formaldehyde Standard, it is primarily handled in closed systems or with proper PPE for protection from formaldehyde when sampling, cleaning or connecting lines.

Several fate and toxicity studies have been conducted on the components of methoxymethanol (methanol and formaldehyde). These studies are briefly reviewed in this testing rationale document, which describes how they meet the SIDS (Screening Information Data Set) end-points of the United States Environmental Protection Agency (USEPA) High Production Volume Challenge (HPV) program. Robust summaries have been prepared for key studies; supporting studies are referenced in these summaries or given as shorter summaries using the IUCLID format. The available data set satisfactorily fulfills the data requirements for the EPA HPV Program. Although all endpoints are not filled by direct experimental data on the methoxymethanol molecule, use of data from formaldehyde and methanol is justified as methoxymethanol is in equilibrium with these species. In addition, estimation of some endpoints from SAR relationships is necessary (due to the instability of the methoxymethanol molecule) and satisfactory to define the hazard of this mixture.

Chemistry of Methoxymethanol

It is necessary to understand some basic information about the chemistry of formaldehyde in water and alcoholic solutions to evaluate the hazards of what is known as "methoxymethanol".

Pure formaldehyde, HCHO, is a gas under normal temperatures and pressures. Commercial formaldehyde is produced as solutions in water and/or lower alcohols. Formaldehyde exists in water as an equilibrium distribution of a homologous series of hydrates such as:

Formaldehyde hydrate	HO-CH2-OH	"Methylene glycol"
Formaldehyde dimer hydrate	HO-CH2-O-CH2-OH	-
Formaldehyde trimer hydrate	HO-CH2-O-CH2-O-CH2-OH	-

etc.

In methanol, formaldehyde primarily exists as an equilibrium distribution of a homologous series of hemiacetals such as:

Formaldehyde hemiacetal	CH3-O-CH2-OH	"Methoxymethanol"
Formaldehyde dimer hemiacetal	CH3-O-CH2-O-CH2-OH	-
Formaldehyde trimer hemiacetal	CH3-O-CH2-O-CH2-O-CH2-OH	-

……..etc.

The length of these oligomers also depends on the relative proportion of formaldehyde to methanol in the mixture.

Commercially prepared aqueous formaldehyde solutions always contain a small amount of methanol (formaldehyde is produced by the partial oxidation of methanol over a metallic catalyst). In many cases, additional methanol is purposely added to the aqueous solutions as a stabilizer to allow storage at lower temperatures. Commercially prepared alcoholic solutions of formaldehyde typically contain water. For example, in Methyl Formcel®, the nominal composition is 55.0% formaldehyde, 34.5 % methanol, and 10.5% water.

Any solution containing formaldehyde, water, and methanol will be a complex equilibrium mixture of these homologous series of hydrates and hemiacetals with small amounts of "free" or uncombined formaldehyde, water, and methanol. Methoxymethanol is just one of the many equilibrium species present. Changing the temperature of the solution, the solution pH, or the concentration of any of the components will shift the equilibrium. Thus, the amount of methoxymethanol present in a formaldehyde solution is dependent not only on the nominal concentrations of formaldehyde, methanol, and water present, but the temperature and pH as well.

Maiwald, et al (2) recently studied the equilibra of these ternary mixtures of formaldehyde-water-methanol as a function of temperature between 298 and 383 K. using quantitative ^{13}C-nmr spectroscopy. They reported that in these mixtures, formaldehyde is predominantly bound in methylene glycol, poly(oxymethylene) glycols,

hemiformal, and poly(oxymethylene) hemiformals. The reader is referred to this article for an in-depth discussion of the ratios of various species as a function of temperature. Some data were extracted from the article as most useful in the current discussion about the actual nature of the methoxymethanol tested and used in industry and is presented below.

In these nmr studies (2), six ternary mixtures containing different ratios of formaldehyde, methanol and water were studied at three temperatures in two sets of independent studies in two different laboratories. As the reproducibility between laboratories was excellent and as the temperature effects were not large, the data from one laboratory at one temperature (298 °K) using two mixtures was selected for presentation. The table below gives the composition of the two mixtures studied along with the composition of the material that was tested in animals for health effects (Japanese material) and the nominal composition of the predominant commercial product in the United States (Celanese material).

Component	Materials used for nmr studies		Experimental and commercial materials	
	Mix A	Mix B	Japanese	Celanese
HCHO (mole fn)	0.2746	0.396	0.223	0.525
H_2O (mole fn)	0.6153	0.0962	0.137	0.167
MeOH (mole fn)	0.1101	0.5078	0.639	0.308

Table 1. Composition of model and commercial mixtures

From the table it can be seen that the commercial material (Celanese) is considerably higher in formaldehyde content than the other mixtures. Although the commercial material is beyond the range for interpolation of approximate quantities of the various species, the NMR data give a good indication of the actual species that exist in solution. The commercial mixture, having a higher level of formaldehyde, might be anticipated to have more F2, F3, F2Me and F4Me components and probably some F5 and F5Me and greater chain length oligomers (see table below for definition of the components). Results of the NMR determination of the relative concentrations of species are shown in Table 2.

Species		Mixture A		Mixture B	
		Peak area	Mole %	Peak area	Mole %
HO-C-OH	F1	0.1469	21.3%	0.0024	0.3%
HO-C-O-C-OH	F2	0.1699	24.7%	0.0025	0.3%
HO-C-O-C-O-C-O-H	F3	0.03	4.4%	nd	nd
HO-C-O-Me	F1Me	0.2327	33.8%	0.708	83.5%
HO-C-O-C-O-Me	F2Me	0.0805	11.7%	0.1169	13.8%
HO-C-O-C-O-C-O-Me	F3Me	0.0286	4.2%	0.0178	2.1%

Table 2. Speciation of model systems

Examination of these data suggests that methoxymethanol is a predominant component of the mixture under conditions of reduced water content and as water content increases (conditions as in the body or the environment), methoxymethanol is rapidly equilibrated into hydrated formaldehyde (F1) and hydrated formaldehyde dimer (F2). This shift in equilibrium is inferred as rapid since these experiments were conducted by mixing two binary mixtures (formaldehyde and methanol, formaldehyde and water) together and recording the spectrum soon thereafter.

Pure (100%) methoxymethanol is unstable at ambient temperatures and will readily form its own equilibrium distribution of hemiacetals, methanol, and formaldehyde. To our knowledge, pure material cannot be purchased commercially or purchased from laboratory supply houses. The limited numbers of studies that have been conducted to study the properties of pure methoxymethanol have used extraordinary conditions to isolate and stabilize the pure material for study (3, 4, 5).

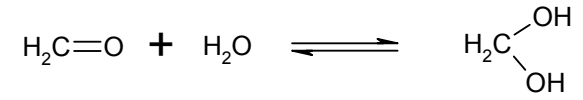
The commercial product used as a source of formaldehyde for production of resins contains as much formaldehyde as is practical, because 1.) Pure formaldehyde is difficult to transport. 2.) The maximum amount of formaldehyde that can be prepared and stored in water at ambient temperatures is about 37% formaldehyde. 3.) Some applications for formaldehyde require removal of excess water and/or addition of methanol.

The rational for selecting the composition used in the SIDS health effects studies of methoxymethanol as conducted by the Mitsubishi Chemical Safety Institute is not available. Our understanding is that Japan had volunteered to sponsor methoxymethanol in the OECD SIDS program and had conducted the basic health effects testing before withdrawing their sponsorship of the material.

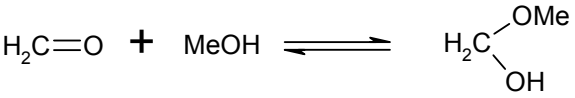
Dealing with the complexity of a ternary system containing a dozen or more oligomeric components may be a practical and realistic way to examine the bulk material relative to its physical and chemical properties (e.g. vapor pressure and boiling point); however, it becomes impractical when considerations of systemic biological activity and environmental distributions under dilution conditions are important. Because of the dynamic equilibrium present, the mass action of biological or environmental water will effectively drive the dissociation of methoxymethanol into methylene glycol, formaldehyde and methanol. In the presence of tissue, protein or dissolved organic matter, free formaldehyde will be removed from the system by covalent reaction resulting in a lowering of the concentration of all these equilibrium species. Never the less, characterization of the mixture under exposure-like conditions will increase understanding of the materials hazard.

Actual measurement of these species when diluted in an aqueous environment is hampered by the low sensitivity of the preferred nmr technique. As the important equilibrium constants for the reaction and dissociation of formaldehyde with water and methanol have been measured, and as oligomer (polymethylene glycol) content falls to insignificant levels under dilution conditions (*vide post*), the ratios of free formaldehyde, methylene glycol and methoxymethanol can be estimated by calculation. Equilibrium constants and methods for calculation of the relative ratios of these species have been published by Hahnenstein et al. (6). The equilibrium constant for the

reaction of formaldehyde with water to give methyleneglycol is temperature dependent and is defined by the equation lnK = -1.902 + 3512/(T/K) and the equilibrium constant for the reaction of formaldehyde with methanol to give methoxymethanol is lnK = -2.325 + 2579/(T/K). These are the primary reactions and constants that describe the interaction of formaldehyde with water and water-methanol mixtures.

A  lnK = -1.902 + 3512/(T/K)

Methylene glycol

B  lnK = -2.325 + 2579/(T/K).

Methoxymethanol

The actual equilibrium concentration of methoxymethanol in dilute aqueous systems is a function of the methanol concentration as shown below. Relative to these calculations, the formaldehyde concentration in this expression [HCHO] refers to the total formaldehyde in the system, which are considered both the free and hydrated forms as there is facile interconversion and the calculation will provide a "worst case" estimate of methoxymethanol in the system. The formulas below describes the equilibria between methoxymethanol (MM), formaldehyde (HCHO) and methanol (MeOH), and methylene glycol (MG), formaldehyde and water (HOH) in the absence of any other reactants for formaldehyde. K_m and K_w are the equilibrium constants for methanol and water, respectively.

$$[MM] = Km\,[MeOH]\,[HCHO]$$

$$[MG] = Kw\,[HOH]\,[HCHO]$$

Combining these equations by substituting for formaldehyde in the first equation gives the relationship between the products:

$$[MM] = \frac{Km}{Kw}\,\frac{[MeOH]\,[MG]}{[HOH]}$$

In dilute aqueous solution, based on the value of K_w and the law of mass action, the methylene glycol concentration is approximately equal to the total concentration of formaldehyde [TF] in aqueous solution. Although this is not exact, it is a reasonable approximation and will slightly overestimate the relative amount of methoxymethanol in the mixture.

$$[MM] \approx \frac{Km}{Kw}\frac{[MeOH][TF]}{[HOH]}$$

Alternatively, by substitution and rearrangement, the concentration of methylene glycol can be related to the concentration of water, methanol and total formaldehyde, if one assumes that the free formaldehyde concentration is insignificant relative to the methylene glycol concentration (a valid assumption for aqueous solutions of methoxymethanol up to 60,000 mg/L) by the equation below to give a more exact calculation.

$$[MG] = \frac{[TF]}{\frac{Km}{Kw}\frac{[MeOH]}{[HOH]} + 1}$$

Using this set of equations and the established values of K_m and K_w, the speciation of these substances when diluted in biological systems (experimental animals or man) or in the environment at relevant concentrations can be estimated with reasonable certainty. The following four tables give the results of these calculations (converted to the more familiar units of mg/L) for four concentrations of the Celanese commercial product and the Japanese test substance in water at two relevant temperatures (20 and 37°C).

Celanese Material at 20°C				
Nominal concentration Mg/L	MeOH mg/L	Methylene glycol mg/L	Free formaldehyde mg/L	Methoxymethanol mg/L
10,000	3051	8210	0.14	765
1000	340	874	0.015	8
100	34	88	0.0015	0.08
10	3	8.8	0.00015	0.0008

Celanese Material at 37°C				
Nominal concentration Mg/L	MeOH mg/L	Methylene glycol mg/L	Free formaldehyde mg/L	Methoxymethanol mg/L
10,000	3112	8300	0.24	646
1000	341	874	0.025	6.8
100	34	88	0.0025	0.07
10	3	8.8	0.00025	0.0007

Japanese Test Material at 20°C				
Nominal concentration Mg/L	MeOH mg/L	Methylene glycol mg/L	Free formaldehyde mg/L	Methoxymethanol mg/L
10,000	6678	3210	0.056	607
1000	696	363	0.006	6.9
100	70	37	0.0006	0.07
10	7	3.7	0.00006	0.0007

Japanese Test Material at 37°C				
Nominal concentration Mg/L	MeOH mg/L	Methylene glycol mg/L	Free formaldehyde mg/L	Methoxymethanol mg/L
10,000	6724	3280	0.09	518
1000	696	363	0.01	5.7
100	70	37	0.0010	0.06
10	7	3.7	0.0001	0.0006

Table 3. Concentrations of Chemical Species at Four Dilutions and Two Temperatures

Some things are apparent from examination of the data in Tables 2 and 3. First, data in table 2 indicate that the equilibrium constant for formation of methoxymethanol from formaldehyde and methanol is greater than that for formation of methylene glycol from formaldehyde and water. This is indicated by the equilibrium ratio of methoxymethanol to methylene glycol (about 2 to 1) in Mixture A when the ratio of methanol to water was about 1 to 5. This is confirmed by the measured equilibrium constant of methoxymethanol being about 40 times as large as that for methylene glycol. More importantly, under environmentally relevant concentrations and in the range of the EC_{50} for formaldehyde, the concentration of methoxymethanol is less than 1% of the methylene glycol concentration. This information in combination with the ECOSAR modeling (which indicates that the hemiacetal methoxymethanol has little intrinsic activity toward fish and invertebrates) strongly indicates that the methoxymethanol content of dilute aqueous solutions is toxicologically inconsequential and need not be taken into consideration relative to the toxicity of these mixtures to fish and invertebrates. In addition, the toxicity of methanol to fish and invertebrates is very low as compared to the aquatic toxicity of formaldehyde (7). Therefore, it can be concluded that use of existing formaldehyde data to define the aquatic hazard of this commercial product is justified.

In the repeated-dose (OECD 422) study reported herein, the high dose was 300 mg/kg-day and the low dose was 12 mg/kg-day. At a dosing volume of 5 ml/kg, the high-dose dosing solution would contain 60 g/L test substance. Due to dilution with water and resulting shift in equilibrium, the formaldehyde content of the high-dose dosing solution exists as about 47% methoxymethanol (mole%) as administered (with about half the methoxymethanol existing as 2-5 unit hemiformal oligomers at this concentration) with almost all of the remainder formaldehyde in the form of methylene glycol and oligomers. The low dose is nominally 12 mg/kg-day, when dosed at this

concentration in water, formaldehyde in the dosing solution is only about 3 mole% in the form of methoxymethanol (with about 2% of that in hemiformal oligomers) and about 96 mole% in the form of methylene glycol. After absorption and distribution in the system circulation, the fraction of the test substance that is methoxymethanol will be reduced by dilution in blood and tissue water to a lower level and by hydrolysis. As a worst case, assuming the blood volume of a 250 g rat to be 15.77 ml (8) and 100% absorption, the maximum blood concentration of the test material mixture at the high dose would be 4800 mg/L. At the high-dose level, methoxymethanol molecules in the systemic circulation represents only about 2.5% (by weight or 1.2 mole %) of the administered test material and the quantity of methoxymethanol in blood decreases exponentially with decreasing doses such that the potential to have significant circulating levels of methoxymethanol at the low dose is essentially nil. The calculations show that if the Celanese commercial material had been used to prepare the dosing solutions, the blood levels of methoxymethanol would be similar at all dose levels in spite of the different ratio of methanol:formaldehyde:water. In either case and at any dose, the blood concentration of methoxymethanol will be very low as compared to the blood concentrations of methanol and methylene glycol. Table 5 shows the calculated maximum blood levels for methylene glycol, methoxymethanol and free formaldehyde as a function of administered dose for the Japanese test material. The second part of this table gives the hypothetical blood levels of these three materials if commercial Celanese "methoxymethanol" were dosed to rats at the same levels.

Supporting the calculations that formaldehyde (or its hydrated and oligomeric forms) is the material that determines the toxic effects of methoxymethanol regarding mammalian toxicity, Johannsen et al. found the LOAEL for formaldehyde to be 100 mg/kg-day in a drinking water study (9). This LAOEL, and the effects reported in the formaldehyde are similar to the LOAEL reported for the repeated dose (OECD 422) study described in the robust summary for methoxymethanol administration. Based on typical parameters, the high dose in this study (150 mg/kg) would entail administration of a solution of approximately 2500 mg/L formaldehyde in drinking water. This concentration of formaldehyde is similar to the dose levels used in the gavage study and such a concentration would contain the formaldehyde primarily as methylene glycol with only about 10% being free formaldehyde. Although one study was drinking water and the other gavage the similarities between the dosing solution concentrations, the effects and the LOAELs/NOAELs suggest that the mammalian toxicity of methoxymethanol is primarily a function of the formaldehyde content of methoxymethanol and the metabolism of methanol to formaldehyde.

			Dosing Solution Speciation as Administered			
Material	Dose mg/kg	Dosing Solution mg/L	Methanol mg/L	Methylene glycol mg/L	Methoxymethanol mg/L	Free formaldehyde mg/L
Japanese Test Mixture	300	60000	31413	11755*	13335*	0.20
	60	12000	7977	3756	852	0.066
	12	2400	1663	853	39	0.015
Celanese Commercial Material	300	60000	15482	36849*	20603*	0.64
	60	12000	3931	9719	1086	0.17
	12	2400	820	2076	46	0.04
* = Includes significant portion in oligomeric form						

Table 4 Speciation and Relative Quantities of Components in Dosing Solutions

Relative to both hazard and risk assessment, likely human exposures are going to be lower than the low dose for rats (12 mg/kg – approximately 840 mg for 70 kg human). This means that regardless of the methoxymethanol formulation, systemic exposure to methoxymethanol molecules will be negligible compared to methylene glycol and methanol at any realistic human exposure level. It appears, therefore, that animal data on formaldehyde and methanol would be more appropriate than actual methoxymethanol data to base hazard and risk assessments relative to oral exposure in man (due to the fact that extrapolation of potential health effects to lower doses should consider that lower doses of methoxymethanol contain almost no methoxymethanol molecules). Even if there were dermal exposure to a solution with a high concentration of methoxymethanol, dilution in the blood and rapid (see kinetics discussion below) establishment of new equilibrium conditions would result in negligible systemic exposure to methoxymethanol.

		Maximum Initial Blood Levels of Each Component			
Material	Administered Dose mg/kg	Methanol mg/L	Methylene glycol mg/L	Methoxymethanol mg/L	Free formaldehyde mg/L
Japanese Test Mixture	300	3290	1668	126	0.047
	60	668	349	5.3	0.0099
	12	134	71	0.21	0.002
Celanese Commercial Material	300	1575	4105	153	0.12
	60	328	840	6.3	0.023
	12	66	169	0.25	0.005

Table 5. Speciation and Relative Quantities of Components When Diluted Into Blood

The above arguments assume that the kinetics of interconversion are relatively rapid. It has been shown that the hydrolysis reactions occur relatively quickly with the pseudo-first-order rate constant for the reaction of methoxymethanol with water initially determined to be 2.6 X 10^{-3} sec^{-1} at 20°C (10). Applying the usual formula, this calculates to a half-life in water of 4.3 minutes. Additional determinations of the hydrolysis rate of methoxymethanol have provided similar results and are given in the "stability" section of this test plan. As oligomeric forms of methoxymethanol are present when the concentrations of methanol and formaldehyde are high, the hydrolysis rates of these species are also relevant. Experimental evidence shows that dilution of methoxymethanol with methanol results in density changes reflective of oligomer methanolysis that are first order and demonstrate a half-life of about 10 minutes at 50°C. The rates of methoxymethanol formation from formaldehyde has also been measured as a function of pH and temperature (6) and a typical rate constant at 30°C and pH 7 is 2 X 10^{-4} sec^{-1} in water-free conditions and 7 X10^{-5} at 20°C, pH 2 and in the presence of 10% mole fraction water. These rate constants correspond to half-lives of about 60 minutes and 160 minutes, respectively. Thus, the rate of methoxymethanol hydrolysis is about an order of magnitude faster than its rate of formation and the hydrolysis rate is fast enough to support the assumption that new equilibrium conditions will be rapidly achieved such that only the equilibrium under diluted conditions need to be considered for most hazard and risk assessments.

In conclusion, methoxymethanol solutions can be accurately characterized with regard to content of the methoxymethanol molecule, methylene glycol, formaldehyde and oligomers based on the extensive studies that have been done to determine the equilibrium constants for the various species. Issues concerning slow transformation of methoxymethanol and subsequent significant exposure, have been shown to be moot regarding typical exposure scenarios by characterization of the rate constants for formation, hydrolysis and interconversion of the various species contained in these mixtures. No additional work is necessary regarding chemical characterization of methoxymethanol because almost all of the equilibrium and kinetic constants are well established allowing one to accurately calculate levels of any species at relevant temperatures. Furthermore, what remains after dilution in an organism or the environment is most appropriately described as a mixture of formaldehyde and methanol; therefore, hazard and risk assessments for humans, animals and plants are better considered as exposure to a simple mixture of formaldehyde and methanol.

Metabolism of Methoxymethanol

Although there are no known studies of the metabolism of methoxymethanol, it can safely be assumed that the vast majority of methoxymethanol that enters the body is rapidly hydrolyzed to formaldehyde and methanol. Both of these materials are metabolized readily to formate and, if not excreted as formate, further to carbon dioxide. Some of the material also enter the C-1 metabolic pool and is incorporated physiologically into cellular macromolecules.

METABOLIC PATHWAYS

Methoxymethanol can be viewed as a mixture of two one-carbon compounds with a common ultimate metabolic product. Because of widespread use and exposure, a large body of work has been conducted on the metabolism of methanol and formaldehyde and the differences in metabolism between rodent models and humans. It is established that methanol is initially metabolized to formaldehyde in man principally by the enzyme alcohol dehydrogenase using NAD/H as a cofactor. In rodents, however, the catalase system using hydrogen peroxide as the cofactor is the primary enzyme responsible. The generated formaldehyde has a short half-life both reacting with nucleophilic molecules and being detoxified by the enzyme formaldehyde dehydrogenase to formate. The excess formate is subsequently removed from the body by urinary excretion or folate-dependent metabolism to carbon dioxide.

Although the pathway formally only requires two one-step oxidations, it is much more complicated and has both qualitative and quantitative differences between rodents and primates. The first important difference between primates (including humans) is the enzymology of methanol oxidation. In man, this oxidation is primarily completed by alcohol dehydrogenase using the plentiful NAD as the electron acceptor. In rats and mice the enzyme responsible for this oxidation is catalase, which relies on a limited flux of hydrogen peroxide as an electron acceptor. This is depicted in Figure 1.

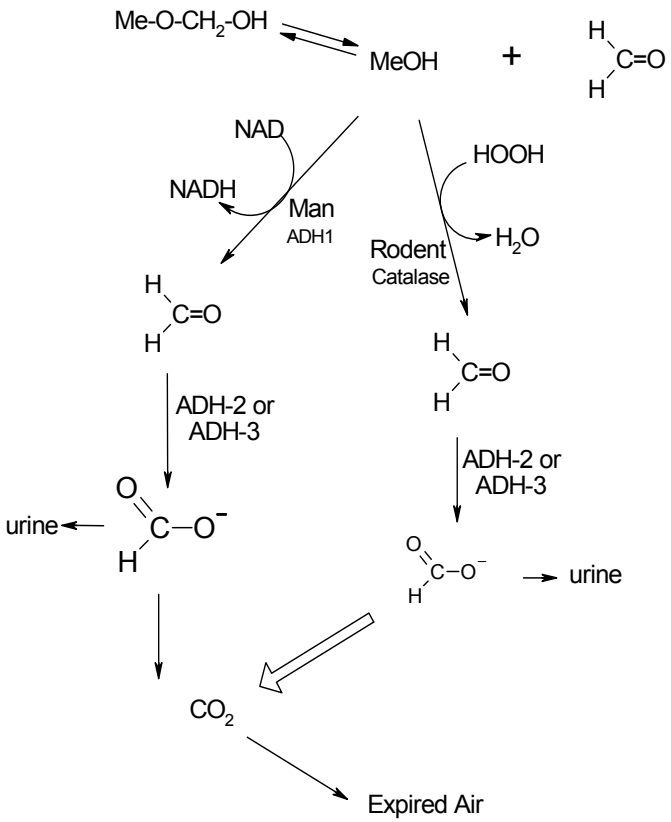


Figure 1. Metabolic Pathways for Methoxymethanol

Studies indicate the catalase pathway becomes saturated at high methanol concentrations (11) and is the rate-limiting step in rodent metabolism of methanol at high doses. In man the rate-limiting step has been shown to be oxidation of formate to carbon dioxide. Thus, at high doses of methanol rats and mice accumulate methanol while humans accumulate formate.

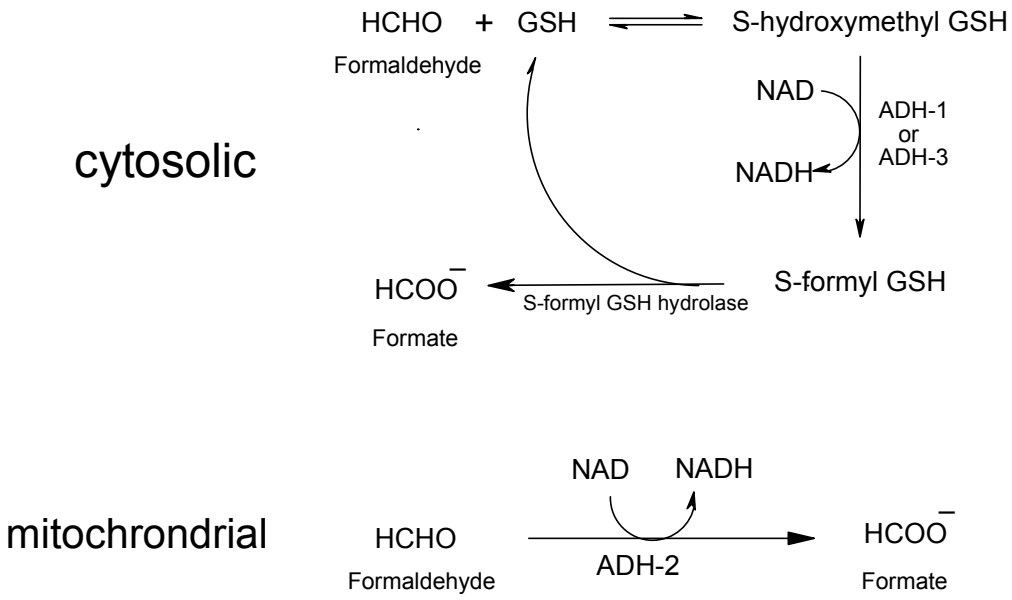


Figure 2. Metabolic Conversion of Formaldehyde to Formate

Conversion of formaldehyde to formate competes with reaction of this substance with nucleophilic centers of cells and tissues. Apparently the formaldehyde dehydrogenase system is very efficient at controlling the level of formaldehyde in the body as the half-life of formaldehyde is given as approximately one minute and the generalization can be made that formaldehyde does not accumulate in humans or experimental animals exposed to methanol (NTP CERHR). The enzyme system known as formaldehyde dehydrogenase is active in both the mitochondria and the cytosol. The cytosolic form is dependent on reduced glutathione to chemically react with cellular formaldehyde and the intermediate product, the S-hydroxymethyl GSH, is oxidized to S-formyl GSH. This S-formyl GSH is broken down into formate and reduced glutathione by S-formyl glutathione hydrolase.

Formate is a relatively unreactive product that is eliminated partly by urinary excretion but, more importantly, can be oxidized to carbon dioxide and eliminated from the lungs with expired air. This oxidation is dependent of tetrahydrofolate and in mediated by two enzymes. The first is formyl THF synthetase that catalyzes the formation

of 10-formyl-tetrahydrofolate from formate and tetrahydrofolate and is dependent on a sufficient supply of tetrahydrofolate. The conversion of 10-formyl-tetrahydrofolate to carbon dioxide and tetrahydrofolate is catalyzed by formyl-THF-dehydrogenase, which also recycles the tetrahydrofolate. Tetrahydrofolate level has been established as the rate limiting cofactor in this oxidation for primates, and as rodents have higher levels of tetrahydrofolate than primates, there is a build up of format in primates that is not typically observed in rats and mice.

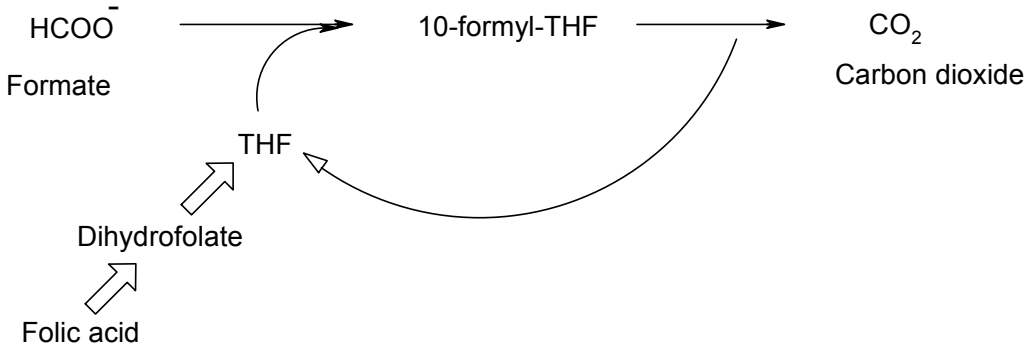


Figure 3. Metabolism of Formate to Carbon Dioxide

It is generally accepted that the known typical human ocular effects (blindness) that result from overexposure to methanol is produced by formic acid (acidosis) and formate toxicity. This effect is not seen in rodents unless the metabolic conversion of formate to carbon dioxide is reduced (e.g. folate deficiency).

These marked metabolic differences between rodents and primates suggest that the rodent is not a satisfactory effects model for methoxymethanol. These differences have also been advanced as reasons to be cautious in hazard assessment of methanol regarding developmental toxicity (11, 12).

Physico-chemical Data

Physical-chemical data for commercial product containing methoxymethanol are available from the manufacturer's information but it must be emphasized that this is a mixture that can vary in composition and definitive physical-chemical data are not appropriate.

	Formcel® Commercial Product ("methoxymethanol")	Formaldehyde	Methanol
Melting Point	NA	-92˚ C (13)	-97.8° C (13)
Boiling Point	90-95°C@1013 hPa (14)	-19.5°C @1013 hPa (13)	64.7°C @1013 hPa (13)
Vapor Pressure	90-95 hPa @ 40˚C (14)	5174 hPa @ 25 (15)	169 hPa @ 25 (15)

Partition Coefficient	Log $K_{o/w}$ = -1.4 (16)*	Log $K_{o/w}$ = 0.35 (17)	Log $K_{o/w}$ = -0.77 (17)
Water Solubility	Soluble in all proportions (14)	~ 55 weight %	Soluble in all proportions (13)
* Calculated value for pure methoxymethanol			

Table 6. Physicochemical Data for Methoxymethanol, Formaldehyde and Methanol

The physical properties of the molecular species methoxymethanol can be estimated using EPIWIN. The estimated boiling point is 91°C and the estimated vapor pressure is 43 hPa at 25°C. These measured and calculated properties indicate that methoxymethanol and commercial mixtures of formaldehyde and methanol are volatile liquids with high water solubility. The value of the partition coefficients suggests that all of the major components will partition preferentially into water and have little potential for bioaccumulation.

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints.

Environmental Fate and Pathways

Biodegradation potential was determined using various methods for both formaldehyde and methanol. There is a solid body of experimental evidence indicating that both can be considered readily biodegradable according to the OECD criteria (18). Considering that methoxymethanol is in equilibrium with formaldehyde and methanol, as methanol and formaldehyde are removed from solution by biodegradation the equilibrium drives hydrolysis of more methoxymethanol to formaldehyde and methanol, especially in dilute aqueous solutions. Thus, even if bacteria do not effectively attack the methoxymethanol molecule, its biodegradation will be facile due to its equilibrium with readily biodegradable formaldehyde and methanol. The existence of a variable quantity of paraformaldehyde in the mixture is another consideration. As paraformaldehyde breaks down in water solution to formaldehyde (19), it will be biodegraded readily after hydrolysis. The breakdown of paraformaldehyde will in turn be dependent on its initial concentration, dissolution rate, temperature and pH. For the purpose of the HPV program, its contribution to the fate of methoxymethanol preparations is suffiently understood.

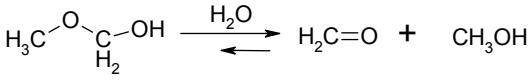
Photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals and was used to estimate the indirect photodegradation rates of the mixture. Direct photolysis, however, was considered first as it is important when it can occur. Before conducting the calculations, consideration has to be given to the nature of the material in the atmosphere.

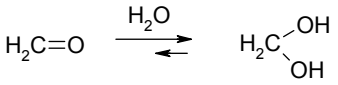
As we are dealing with variable composition commercial mixtures and assuming the vapor phase chemistry of the various components is similar to the liquid phase chemistry, it can be surmised that there will be four primary

components introduced into the atmosphere resulting from the use of commercial methoxymethanol. These are methoxymethanol, formaldehyde, methanol and water. An important secondary component would be the hydrated form of formaldehyde (dihydroxymethane) resulting from the reaction of formaldehyde with atmospheric water vapor. In liquid phase this hydration is a very facile reaction with an equilibrium constant greater than 1000 favoring the hydrated form (20). Formation of polymeric forms of formaldehyde in the atmosphere is not considered important due to dilution effects in the vapor phase; however, sublimation of oligomeric formaldehyde from spills of commercial methanol-hydrated-formaldehyde is possible. The APOWIN calculation (shown in the robust summaries) indicates that hydrogen abstraction is very a favorable process for reaction of oligomeric formaldehyde with hydroxyl radical and oligomers will only have an atmospheric half-life of approximately two hours. Thus, as oligomers are expected to contribute little to the quantity of material in the air and will not contribute to an extended half-life, they can be ignored relative to atmospheric photodegradation.

Regarding direct photolysis, it can be surmised by inspection of the possible species formed in the atmosphere that none of these has a chromophore that absorbs light above 295 nm (the approximate cut off for light energy transmitted to the troposphere). Direct photolysis, therefore, is considered unimportant.

In figure 4 the chemistry of methoxymethanol in the atmosphere is shown with half-lives for photodegradation that were calculated by APOWIN.





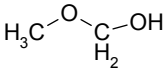
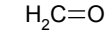
SPECIES	HALF-LIFE IN AIR
 Methoxymethanol	6.1 hours
 Formaldehyde	15.8 hours
 Methylene Glycol (Formaldehyde Hydrate)	10.9 hours
 Methanol	11.3 hours

Figure 4. Atmospheric Chemistry and Photodegradation

APOWIN estimates the rate constant for reaction of a substance with hydroxyl radicals that formed in the troposphere. The program uses the estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. The program produced an estimated rate constant for each atmospheric component. Using the default atmospheric hydroxyl radical concentration in APOWIN (1,500,000 molecules/cc) and the estimated rate constant for reaction of each material with hydroxyl radical, the estimated half-life of each component was calculated. These half-life values are shown in Figure 4. Because methoxymethanol as produced and used (methanol-hydrated-formaldehyde) has a variable composition, and as the amount of atmospheric water vapor affects the chemistry, only a range of half-live values can be given. Fortunately, all components have similar hydroxyl radical reaction rate constants and the range is reasonably narrow. The half-life range is estimated to be 6.1 to 15.8 hours (see accompanying robust summary).

Water stability has been quantitatively determined for methoxymethanol in a series of kinetic studies. The second order rate constants for reaction of methoxymethanol with water, hydrogen ion and hydroxide ion have been determined at 25°C (21). To estimate the half-life in water at various pH levels, these second-order rate constants were converted to pH specific pseudo first order rate constants and the half-lifes calculated for the water, the hydrogen ion and the hydroxyl ion reaction at several pH levels. From these calculations, the half-life of methoxymethanol at various pH levels can be determined. As there is also a dependency of the hydrolysis rate on ionic strength and the nature of the solutes in natural waters, these half-lives must be viewed as approximations of the actual hydrolysis rate under environmental conditions. The maximum half-life is about six minutes and is shortened by acidic or basic conditions but displays a broad peak (see robust summary for actual rate constants).

pH	$T_{1/2}$ Water	$T_{1/2}$ H+	$T_{1/2}$ OH^-	Approximate overall half-life
2	6 min	2 min	810 hours	2 min
4	6 min	3.3 hours	81 hours	6 min
6	6 min	333 hours	490 min	6 min
7	6 min	>1000 hours	49 min	6 min
8	6 min	>1000 hours	4.9 min	5 min
9	6 min	>1000 hours	30 sec	30 sec

Table 7. The Half-life of Methoxymethanol in Water at Several pH Values

Methanol is a simple alcohol and alcohols are one of the chemical groups considered stable to hydrolysis (22). Methanol, therefore, is considered to be a water stable component of this mixture.

Formaldehyde is known to be water reactive reversibly forming a hydrate (HO-CH2-OH) the equilibrium constant for formaldehyde hydrate formation is > 1000 (23). Thus, formaldehyde is known to be stable indefinitely in water existing 99.9% as a hydrated species.

Theoretical Distribution (Fugacity) of methoxymethanol in the environment is an extremely complicated matter due to the atmospheric chemical reactions and equilibrium of methoxymethanol with formaldehyde and methanol that the equilibrium between formaldehyde and its hydrate. These equlibria are dependent on atmospheric water vapor concentration and temperature.

To simplify matters a Level 3 fugacity model was run on each major component independently using the MacKay model with standard defaults in EPIWIN v 3.05. Actual physical properties were used for methanol and formaldehyde. Because methoxymethanol, to our knowledge, has never been studies in the pure bulk liquid form, the EPIWIN calculated values were accepted for the fugacity calculation.

Media	Methoxymethanol	Methanol	Formaldehyde
o Air	1.92%	13%	2.7%
o Water	54.8%	47.2%	51.3%
o Soil	43.2%	39.7%	45.9%
o Sediment	0.0913%	0.0705%	0.0871%

Table 8. Level 3 Fugacity Calculations

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints.

Ecotoxicity

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to aquatic species (39), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to aquatic species, formaldehyde is the species that will determine the acute ecotoxicity of this mixture. In recognition of this, the robust summaries flagged as "critical for SIDS endpoint" have been adopted from the formaldehyde SIDS document. The values for formaldehyde were adjusted to account for the 55% concentration of formaldehyde in commercial product and the adjusted vales are presented below. Full details and robust summaries for the critical studies are presented in the attachment (Robust Summary Document). The reader is also referred to the formaldehyde SIDS document (24) for more supporting studies.

Acute Aquatic Toxicity of Methoxymethanol Based on the Toxicity of Formaldehyde	
Fish, 96 hour LC_{50}	ca. 45 mg/L
Daphnia, 48 hour EC_{50}	ca. 10.5 mg/L
Algae, 72 hour EC_{50}	ca. 11.5mg/L

Table 9. Acute Aquatic Toxicity of Methoxymethanol

As part of the estimation procedure, to gain some assurance that the methoxymethanol molecule did not have higher toxicity potential that formaldehyde, ESOSAR estimates for the toxicity of methoxymethanol were made using the neutral organics model. It should be noted that it is recognized that these estimates are not valid for estimation of the toxicity of methoxymethanol in water since this hemiacetal will hydrolyze in water to methanol and methylene glycol. These estimated using the neutral organics model are presented below:

Species	Duration	Endpoint	Prediction mg/L
Fish	96-hr	LC_{50}	72256
Fish	14-day	LC_{50}	76256
Daphnid	48-hr	LC_{50}	61217
Green Algae	96-hr	EC_{50}	31468
Fish	30-day	ChV	5381
Daphnid	16-day	EC_{50}	709
Green Algae	96-hr	ChV	441
Fish (SW)	96-hr	LC_{50}	3197
Mysid Shrimp	96-hr	LC_{50}	236000
Earthworm	14-day	LC_{50}	4256

Table 10. ECOSAR Predictions for Methoxymethanol Molecular Species

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints without unnecessary aquatic animal usage.

Health Effects

Limited health effects studies that were designed specifically to fill the SIDS HPV endpoints have been conducted using methoxymethanol. These studies were recently conducted at the Kashima Laboratory of the Mitsubishi Chemical Safety Institute Ltd. (25). These studies used a material that was 46.7% HCHO with 44.93% Methanol (essentially a two to one molar ratio of methanol to formaldehyde).

In addition, a very comprehensive set of studies has been conducted to determine the potential health effects of both formaldehyde and methanol. From a health effects data view, these are two of the best-studied materials in commerce. As methoxymethanol will readily breakdown in the environment and the body, formaldehyde and methanol data are also relevant to the toxicity of this commercial material.

NOTE: In the following discussion the term "methoxymethanol" will generally be used to refer to the materials actually being tested but should be kept in mind that the methoxymethanol molecule is in dynamic equilibrium with formaldehyde, methanol and water.

Acute Toxicity

Oral Exposure

A modern guideline or guideline-like oral-gavage study has been completed by the Kashima Laboratory of the Mitsubishi Chemical Safety Institute Ltd. (26). This study used a test material that was 46.7% methoxymethanol with 44.93% Methanol and the remainder presumed to be water. The study found the oral LD_{50} values for male or female rats were 1269 mg/kg for males (95% confidence limit: 981-1636 mg/kg), and 1451 mg/kg for females (95% confidence limit: 1059-2000 mg/kg). Full details are given in the accompanying robust summary.

The acute oral LD_{50} of formaldehyde has been reported to be 600-700 mg/kg by Tsuchiya K. *et al.* (27) and 800 mg/kg by Smyth *et al*. (28). In the studies, Wistar rats were treated by gavage with 2 or 4 % formaldehyde solutions (formaldehyde with or without methanol stabilization). No relevant differences in toxicity were observed with regard to the additional methanol. Lethality occurred mainly during the first day after administration. Signs of toxicity were not reported.

The acute oral toxicity of methanol to rats has been reported several times in the literature. LD_{50} values of 5628 mg/kg (29), 9100 mg/kg (30), 9470 mg/kg (31), 11520 mg/kg (32), and 12750 mg/kg (33) have been reported. Although many of these reports lack details, the approximate range is consistent.

The oral LD_{50} of methoxymethanol as prepared in a methanolic solution was found to be about 1350 mg/kg. As discussed in the chemistry section, this mixture is about 25% formaldehyde. If the LD_{50} is corrected to a

formaldehyde basis, the corresponding LD_{50} would be 350 mg/kg. As the reported oral LD_{50} for formaldehyde is 600-800 mg/kg, this implies that there is some additional acute toxicity due either to the methanol or the existence of the formaldehyde as methoxymethanol. As the acute toxicity of methanol is an order of magnitude less than that of methoxymethanol mixture (as tested) or formaldehyde in aqueous solution and as methanol is metabolically converted to formaldehyde and then formate, it seems unlikely that there is a joint systemic toxic action of formaldehyde and methanol. It is speculated that the presence of excess methanol in the gavage solution has a stabilizing effect on the formaldehyde, which reduces its covalent bonding to the gastric mucosa and facilitates systemic absorption of formaldehyde. Although this is purely speculation, it is consistent with the known acute oral toxicities of formaldehyde and methanol (including the metabolism of methanol to formaldehyde), the known high reactivity of formaldehyde, the known stabilizing effect of methanol on formaldehyde solutions and the known chemical equilibrium state of ternary mixtures of formaldehyde, methanol and water (*vide ante*).

What is clear is that the rat oral LD_{50} of methoxymethanol as prepared is not greatly different from what would be predicted by considering methoxymethanol to be a simple mixture of formaldehyde, methanol and water. Based on this knowledge, the approximate LD_{50} of any commercial ternary mixture of formaldehyde, methanol and water (the commercial product know as methoxymethanol) can be predicted based on the formaldehyde content and adding an uncertainty factor in the range of two to account for either joint toxic action or facilitated absorption.

Rat Acute Oral Toxicity Data			
Material	**LD_{50} (mg/kg)**		**Reference**
	Male	Female	
Methoxymethanol with Methanol	1269	1451	26
Formaldehyde	600-800 mg/kg		34
Methanol	5,600-12,000 mg/kg		29, 30, 31, 32, 33

Table 11. Acute Oral Toxicity Data

Inhalation Exposure

No data were found for the inhalation toxicity of methoxymethanol. From a practical viewpoint, this material will dissociate into formaldehyde and methanol in moist air. As it is unstable, formaldehyde acute inhalation toxicity would appear to provide a reasonable surrogate.

The 4-hour acute inhalation LC_{50} of formaldehyde has been reported to be 480 ppm by Nagorny *et al.*(35) and the 4-hour LC_{50} for methanol has been reported as 64,000 ppm (36) and 73,000 ppm (37).

Dermal Exposure

No studies of the acute dermal toxicity of methoxymethanol were found.

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints for acute toxicity. Although this material is variable in composition the high level of acute toxicity for formaldehyde provides a reasonable estimate for oral and inhalation toxicity.

Repeat Dose Toxicity

Oral Exposure

A modern guideline OECD-422 repeated-dose study with a reproductive and developmental screen has been conducted on methoxymethanol by the Kashima Laboratory of the Mitsubishi Chemical Safety Institute Ltd.

This OECD-422 study was conducted using Sprague-Dawley rats the same test material that was employed in the acute oral study and the two genotoxicity studies. The test material was 46.7% methoxymethanol with 44.93% methanol and the remainder presumed to be water. Doses were selected as 12, 60 or 300 mg/kg-day based on a preliminary study. Dosing was started 14 day prior to mating and continued until day four of lactation for the dams (4 to 47 days) and for a total of 44 days for the males. Pregnant females were allowed to litter and the pups were thoroughly examined at littering and on lactation day four, when they were sacrificed. Complete necropsies were performed on parental animals followed by microscopic examination of tissues. Hematology and clinical chemistry studies were also conducted.

The major effect of methoxymethanol on parental animals was severe irritation of the gastric musosa. Which was revealed most clearly by the microscopic examination Changes attributed to administration of the test substance were found in the stomach, duodenum and adrenal glands. Ulceration of the gastric glands and the mucosa of the stomach were noted in 5 males and 8 females in the 300 mg/kg-day group. The ulcerated lesions were swollen with effused inflammatory cells and granulomatous tissue, and there were even cases which had formed either large granuloma or the pathological changes had penetrated through the muscular layer. In addition, an eroded lesion of the gastric gland where only the top layer of the mucosa had been exfoliated was found in 2 males in the 60 mg/kg-day group, and also in 3 males and 2 females in the 300 mg/kg-day group. In the 300 mg/kg-day group, 9 males and 5 females showed an inflammatory cell infiltration extending to the submucosal tissue. Focal regenerative changes of the glandular epithelium of the gastric gland was seen in 3 males in the 60 mg/kg-day group, and in 6 males and 5 females in the 300 mg/kg-day group. The focal regenerative mucosa consisted of

basophilic glandular epithelia different from the normal proper glandular cells. All of these changes were most frequently seen in the proventriculus and the periphery of the gastric gland border. Hypertrophy of the duodenal mucosa was found in 6 males of the 300 mg/kg-day group. The hypertrophied mucosa consisted of deep crypts and tall villi and there was clearly a difference between the duodena of the males in this group and those of controls. Examination of the adrenal glands revealed hypertrophy of zona fasciculata and zona reticularis in 2 males of the 300 mg/kg-day group. These two animals also showed severe ulceration of the stomach.

Hematology revealed changes in RBC's (reduced number), reticulocytes and platelets (increased) that were only seen in the high-dose males. These effects may have been related to gastric ulceration and subsequent loss of blood. Clinical chemistry revealed effects only for the high-dose males. Effects were confined to reduction in total protein and albumin and the albumin/globulin ratio. These effects are consistent with gastric ulceration and subsequent loss of blood and are considered secondary to the gastric lesions.

Effects appear to be primarily at the site of contact and related to the irritant properties of the test substance. The GI tract is identified as the target organ and biochemical and hematologic changes are considered secondary to gastric ulceration and subsequent loss of blood. The LOAEL was determined to be 60 mg/kg-day for males and 300 mg/kg-day for females. The NOAELs are considered to be 12 mg/kg-day for males and 60 mg/kg-day for females.

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints for repeated-dose toxicity.

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening is for two end-points: generally one sensitive to point mutation and one sensitive to chromosomal aberrations. In the case of this material, adequate "*in vitro"* and *"in vivo"* tests have been conducted to cover both of these two endpoints and, in addition, exhaustive genotoxicity studies have been conducted for both Formaldehyde and Methanol.

Genetic Toxicology *in vitro*

A Salmonella reverse mutation assay is available on methoxymethanol and used a material that was 46.7% methoxymethanol with 44.93% Methanol and the remainder presumed to be water. In this study methoxymethanol was found to have activity toward strains TA98 and TA100. The activity of methoxymethanol was marginal but it was strong enough to meet the criteria of a doubling of the number of revertant colonies seen in the controls and a dose-response relationship. As expected for this formaldehyde releasing material, methoxymethanol was toxic to Salmonella at 500 μg/plate and above.

These revertant results are both qualitatively and quantitatively similar to the results obtained by the NTP (38) using formaldehyde. In the NTP study results, toxicity was observed in the range of 166 to 333 μg/plate and the

maximum genotoxic activity was observed at correspondingly lower concentrations than methoxymethanol. This is consistent with the hypothesis that in water solutions the methoxymethanol equilibrium with formaldehyde and methanol strongly favors the hydrolyzed forms of free formaldehyde and methanol.

Studies have shown that methanol is negative in bacterial reverse mutation assays and is essentially non-inhibitory to test Salmonella and *E. coli* (39). These data on methoxymethanol suggest that in aqueous solutions, methoxymethanol acts like a simple Formaldehyde solution and that the methanol (both the stoichiometric excess and that produced by hydrolysis of methoxymethanol) does not influence either the mutagenic properties of formaldehyde toward sensitive bacterial strains nor the cytotoxicity of formaldehyde toward these bacteria.

Genetic Toxicology *in vivo*

Mammalian genotoxicity was assessed "*in vivo*" using Chinese hamster lung (CHL) cells in an *in vitro* chromosomal aberration test with methoxymethanol. The test material was the same that was used for the bacterial reverse mutation assay and was 46.7% methoxymethanol with 44.93% methanol and the remainder presumed to be water.

After exposing CHL cells for 24 hours to methoxymethanol, the proportion of cells with chromosomal structural changes and polyploid cells increased significantly in a concentration-related fashion. In the high concentration group (0.020 mg/ml), methoxymethanol was determined to be positive for structural aberrations and equivocal for polyploid cells.

After exposing CHL cells for 48 hours to methoxymethanol the proportion of cells with chromosomal structural changes and polyploid cells were significantly increased and indicated a equivocal result high concentration group (0.020 mg/ml). On the other hand, the high concentration group with metabolic activation with 6-hour exposure showed chromosomal structural aberrations in more than 16% of the cells examined indicating a positive result. The frequency of polyploid cells increased significantly in the medium and high concentration groups indicating equivocal results.

It is concluded that methoxymethanol is positive for producing chromosomal structural aberrations in CHL cells in vitro. This result is similar to that obtained for formaldehyde using CHO cells where it was found to be positive for producing chromosome aberrations in the presence or absence of metabolic activation at concentrations levels similar to those producing positive results for methoxymethanol (40).

Methanol, on the other hand, is considered non-genotoxic and of low cytotoxicity (41). As was the case for the bacterial reverse mutation assays, these data on methoxymethanol suggest that in aqueous solutions, methoxymethanol acts like a simple Formaldehyde solution and that the Methanol (both the stoichiometric excess

and that produced by hydrolysis of methoxymethanol) does not influence either the genotoxic properties of Formaldehyde toward sensitive mammalian cells nor the cytotoxicity of Formaldehyde toward these cells.

Recommendation: The HPV requirement for genetic testing has been met as assays sensitive to both point mutation and to clastogenic effects have been conducted using an acceptable protocol. No additional testing is recommended.

Reproductive Toxicity

Methoxymethanol was tested for reproductive toxicity using a combined repeat-dose, reproductive and developmental toxicity screening study (OECD 422). Please refer to the "repeated dose" section of this document or the accompanying "robust summary" for more information about the study design. All females, where mating was confirmed, became gravid. There was no effect of the test substance on either the mating or fertility indices. Most pairs successfully mated during the first estrous cycle and there were no significant differences among groups on the day of mating. There was no difference between control and dosed groups on any parameter associated with successful mating, gestation or delivery. Histopathological examination of the parental generation revealed no adverse effects on the reproductive organs.

Although this is only considered a screening study, no hints of adverse reproductive effects were reported. In addition, both Formaldehyde and Methanol have been investigated regarding reproductive toxicity. No adverse effects of chronic formaldehyde administration by drinking water on reproductive organs were reported in an 1989 in a chronic study with rats by Til et al. at doses that induced stomach lesions (approx. 82 and 109 mg/kg-day for male and female rats, respectively). Ovaries and testes of a subset of animals (at least 10 animals per dose and gender) were weighed at weeks 53, 79 and 105. Histological examinations of ovaries, mammary glands, uteri and testes, prostate glands, epididymides were performed on all animals of control and high-dose groups. Mammary glands, ovaries and testes of three low- and mid-dose group animals were also examined in week 105 (42).

Potential adverse effects of methanol on reproductive organs were studied by Lee et al. (43), who exposed 8-week-old Sprague-Dawley rats at 200 ppm for 8 hours/day (ca. 37 mg/kg) for 1– 6 weeks and observed no effect on testosterone levels, weights of androgen sensitive organs, capability of in vivo-exposed testes to produce testosterone in vitro; he also reported lack of gross morphological effect on reproductive organs. In addition, normal and folate-deficient, Long-Evans rats exposed to 800-ppm methanol for 20 hours/day (ca. 378 mg/kg-day), 7 days per week for 13 weeks had no adverse findings in the testicular histology at 10 months of age (ibid.). A study reported by Poon et al. (44) reported no lesions in the reproductive organs of 4–5 week-old male and female Sprague-Dawley rats that inhaled 2,500-ppm methanol vapors for 6 hours/day (ca 370 mg/kg-day) for 4 weeks. Overall, the weight of evidence indicates little potential for methanol-induced adverse reproductive effects.

In summary, a combination of studies on methoxymethanol, formaldehyde and methanol indicate no adverse effects on reproduction or reproductive organs.

Recommendation: No additional testing is required as the available data are sufficient to assess the reproductive toxicity of this material.

Developmental Toxicity

Methoxymethanol was tested for reproductive toxicity using a combined repeat-dose, reproductive and developmental toxicity screening study (OECD 422). Please refer to the "repeated dose" section of this document or the accompanying "robust summary" for more information about the study design.

In brief, results of the developmental toxicity aspects of this OECD-422 study showed that no malformations were observed that were attributable to administration of the test substance. High-dose pups were not different from controls in body weight, sex ratio, mean pup weights, number of pups born, or other similar parameters. Skeletal examination of the high-dose and control groups showed no compound-related effects. Visceral examination revealed a significant increase in the occurrence of patent foramen ovale in the 300 mg/kg-day group. This is interpreted as a fetotoxic effect at the high dose associated with a developmental delay. The developmental and maternal NOAEL is considered to be 60 mg/kg-day.

The developmental toxicity of formaldehyde has been studied in an inhalation prenatal toxicity study reported by Martin (45,46). This study indicated no teratogenic effects after inhalation of 2, 5, or 10 ppm (2.4, 6, 12 mg/m3; ca 0.23, 0.65 or 1.3 mg/kg-day) formaldehyde during gestation days 6 - 15 in the rat. There were two control groups in the study, one was sham-treated (air only), and the other was maintained without any treatment in the animal room. At 10-ppm formaldehyde, a significant decrease in maternal food consumption and body weight gain was reported but pregnancy parameters were unaffected. No evidence of maternal toxicity or developmental that was considered related to exposure was found at the lower concentration levels. The maternal NOAEL is 5 ppm and the fetal NOAEL is 10 ppm. These results are supported by a teratogenicity study by Saillenfait et al. reported in 1989 (47), in which higher formaldehyde concentrations (up to 40 ppm, 50 mg/m^3) were used for exposure. At 20 ppm (25 mg/m^3) and above a slight decrease of the fetal weights was observed. These concentrations, however, cause severe irritations of the upper respiratory tract of dams.

Several developmental toxicity studies of methanol have been conducted and it has been reported that methanol is a developmental toxin to rodents at very high doses. In a study reported by Nelson et al. (48) inhalation exposure of Crl:Sprague-Dawley rats to 20,000 ppm methanol vapor for 7 hours/day (ca. 3,300 mg/kg-day) on gestation day 7–15 was associated with prenatal developmental toxicity evidenced by reduced fetal weight, increased litter incidence of exencephaly and encephalocele, and skeletal malformations. This inhalation concentration also caused clinical signs of maternal intoxication in the early days of exposure but no other maternal effects were reported. Developmental toxicity without malformations was also observed following exposure to 10,000 ppm for

7 hours/day (ca. 1650 mg/kg-day) on gestation day 1–19 as evidenced by statistically significant reductions in fetal body weight. The National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (CERHR) panel's review of this study designated 10,000 ppm inhaled methanol as a maternal NOAEL and 5,000 ppm as a fetal NOAEL (49)

In mice, positive studies were reported by Rogers et al. (50). In these studies, exposure of Crl:CD-1 mice to methanol vapor at concentrations of 2,000 ppm or greater for 7 hours/day on gestation day 6 –15 was reported to be associated with developmental toxicity as evidenced by cleft palate, exencephaly and skeletal malformations. The initial appearance of malformations was reported to be cervical ribs seen at 2,000 ppm and cleft palate and exencephaly at 5,000 ppm, and adverse effects on the number of live pups per litter and fetal weight were seen at 7,500 and 10,000 ppm, respectively. No maternal toxicity was apparent at any dose and the developmental NOAEL was considered to be 1000 ppm. Additional studies were conducted by gavage to link the inhalation produces blood methanol levels with blood methanol levels produced by gavage. They established that twice-daily gavage dosing with 2000 mg/kg (4,000 mg/kg-day) produced blood levels similar to those produced by inhalation exposure at 10,000 for 7 hours/day. In addition, this twice daily gavage dosing produced similar a similar pattern of developmental as the 10,000 ppm 7 hour/day inhalation exposure.

It should be kept in mind when evaluating the developmental data relative to human risk that there are multiple differences in the way methanol is detoxified in a rodent verses a human. These differences, discussed in detail in an earlier section of this document, indicate that additional caution should be used when extrapolating rodent data from methanol to humans as findings in rodents may not be directly applicable to man.

Recommendation: No additional testing is required as the available data are sufficient to assess the developmental toxicity of this material.

Conclusions

With regard to the parameters specified in the EPA HPV Challenge program, the available information fills all of the requirements for physicochemical parameters, environmental fate, and toxicity information. No additional testing is recommended.

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